

We claim:

1. A method for identifying a thermostable polymerase having altered fidelity, comprising generating a random population of polymerase mutants by mutating at 5 least one amino acid residue of a thermostable polymerase and screening said population for one or more active polymerase mutants by genetic selection.

2. The method of claim 1, wherein two or more amino acid residues of said thermostable polymerase are 10 mutated.

3. The method of claim 1, further comprising determining a fidelity of said active polymerase mutant.

4. The method of claim 1, wherein said mutated amino acid residue is adjacent to an immutable or 15 nearly immutable residue.

5. The method of claim 4, wherein said mutated amino acid residue is immediately adjacent to an immutable or nearly immutable residue.

6. The method of claim 1, wherein said 20 mutated amino acid residue is in an O-helix of a thermostable polymerase.

7. The method of claim 4, wherein said mutated amino acid residue is adjacent to an amino acid residue corresponding to Arg659, Lys663, Phe667 or Tyr671 25 in *Taq* DNA polymerase.

8. The method of claim 7, wherein said thermostable polymerase is *Taq* DNA polymerase.

9. A method for identifying a thermostable polymerase having altered fidelity, comprising generating a random population of polymerase mutants by mutating at least one amino acid residue in an active site O-helix of 5 a thermostable polymerase and screening said population for one or more active polymerase mutants.

10. The method of claim 9, wherein two or more amino acid residues of said thermostable polymerase is mutated.

11. The method of claim 9, further comprising determining a fidelity of said active polymerase mutant.

12. The method of claim 9, wherein said mutated amino acid residue is adjacent to an immutable or nearly immutable residue.

13. The method of claim 12, wherein said mutated amino acid residue is immediately adjacent to an immutable or nearly immutable residue.

14. The method of claim 12, wherein said one or more amino acid residues is adjacent to an amino acid residue corresponding to Arg659, Lys663, Phe667 or Tyr671 20 in Tag DNA polymerase.

15. The method of claim 14, wherein said thermostable polymerase is Tag DNA polymerase.

16. An isolated thermostable polymerase mutant 25 having altered fidelity, wherein said mutant comprises one or more mutated amino acid residues in the active site O-helix of a thermostable polymerase.

17. The polymerase mutant of claim 16, wherein
said polymerase is *Taq* DNA polymerase.

18. The polymerase mutant of claim 16, wherein
said mutated amino acid residue is adjacent to an
5 immutable or nearly immutable residue.

19. The polymerase mutant of claim 18, wherein
said mutated amino acid residue is immediately adjacent
to an immutable or nearly immutable residue.

20. The polymerase mutant of claim 18, wherein
10 said mutated amino acid residue is adjacent to an amino
acid residue corresponding to Arg659, Lys663, Phe667 or
Tyr671 in *Taq* DNA polymerase.

21. The polymerase mutant of claim 20, wherein
said polymerase is *Taq* DNA polymerase.

15 22. The polymerase mutant of claim 17, wherein
said polymerase mutant is a high fidelity mutant.

23. The polymerase mutant of claim 22, wherein
said polymerase mutant comprises one or more amino acid
substitutions selected from the group consisting of
20 Phe667Leu; Asn666Asp; Asn666Ile; Ile665Leu; Leu670Val;
Arg660Tyr; Arg660Ser; Gly668Arg; Arg660Lys; Gly668Ser;
Gly668Gln; Thr664Ile and Asn666Asp; Ala661Ser and
Val669Leu; Ala661Glu, Ile665Thr, and Phe667Leu; and
Thr664Pro, Ile665Val and Asn666Tyr.

25 24. The polymerase mutant of claim 17, wherein
said polymerase mutant is a low fidelity mutant.

25. The polymerase mutant of claim 24, wherein
said polymerase mutant comprises substitution of one or
more amino acids selected from the group consisting of
Ala661, Thr664, Asn666 and Leu670.

5 26. The polymerase mutant of claim 25, wherein
said polymerase mutant comprises one or more amino acid
substitutions selected from the group consisting of
Ala661Glu; Ala661Pro; Thr664Pro; Thr664Asn; Thr664Arg;
Asn666Val; Thr664Pro and Val669Ile; Arg660Pro and
10 Leu670Thr; Arg660Trp and Thr664Lys; Ala662Gly and
Thr664Asn; Ala661Gly and Asn666Ile; Ala661Pro and
Asn666Ile; and Ala661Ser, Ala662Gly, Thr664Ser and
Asn666Ile.

27. An isolated nucleic acid molecule encoding
15 a polymerase mutant having high fidelity, comprising a
nucleotide sequence encoding substantially an amino acid
sequence of *Taq* DNA polymerase I comprising one or more
amino acid substitutions selected from the group
consisting of Phe667Leu; Asn666Asp; Asn666Ile; Ile665Leu;
20 Leu670Val; Arg660Tyr; Arg660Ser; Gly668Arg; Arg660Lys;
Gly668Ser; Gly668Gln; Thr664Ile and Asn666Asp; Ala661Ser
and Val669Leu; Ala661Glu, Ile665Thr, and Phe667Leu; and
Thr664Pro, Ile665Val and Asn666Tyr.

28. An isolated nucleic acid molecule encoding
25 a polymerase mutant having low fidelity, comprising a
nucleotide sequence encoding substantially an amino acid
sequence of *Taq* DNA polymerase I comprising substitution
of one or more amino acids selected from the group
consisting of Ala661, Thr664, Asn666 and Leu670.

29. The nucleic acid molecule of claim 28, wherein said polymerase mutant comprises one or more amino acid substitutions selected from the group consisting of Ala661Glu; Ala661Pro; Thr664Pro; Thr664Asn; Thr664Arg; Asn666Val; Thr664Pro and Val669Ile; Arg660Pro and Leu670Thr; Arg660Trp and Thr664Lys; Ala662Gly and Thr664Asn; Ala661Gly and Asn666Ile; Ala661Pro and Asn666Ile; and Ala661Ser, Ala662Gly, Thr664Ser and Asn666Ile.

10 30. A method for identifying one or more mutations in a gene, comprising amplifying said gene using a high fidelity polymerase mutant under conditions which allow polymerase chain reaction amplification.

15 31. A method for identifying one or more mutations in a gene, comprising amplifying said gene using the high fidelity polymerase mutant of claim 22 under conditions which allow polymerase chain reaction amplification.

20 32. The method of claim 30, wherein said gene is amplified by exposing the strands of said gene to repeated cycles of denaturing, annealing and elongation to produce an amplified product.

25 33. The method of claim 32, further comprising determining the presence or absence of one or more mutations in the sequence of said gene.

34. The method of claim 30, wherein said polymerase mutant comprises one or more amino acid substitutions selected from the group consisting of Phe667Leu; Asn666Asp; Asn666Ile; Ile665Leu; Leu670Val; Arg660Tyr; Arg660Ser; Gly668Arg; Arg660Lys; Gly668Ser;

Gly668Gln; Thr664Ile and Asn666Asp; Ala661Ser and Val669Leu; Ala661Glu, Ile665Thr, and Phe667Leu; and Thr664Pro, Ile665Val and Asn666Tyr.

35. A method for accurately copying repetitive
5 nucleotide sequences, comprising amplifying said
repetitive nucleotide sequence using a high fidelity
polymerase mutant.

36. The method of claim 35, wherein said
repetitive nucleotide sequence is in a gene.

10 37. The method of claim 35, wherein said
repetitive nucleotide sequence is in a microsatellite
between genes.

15 38. A method for accurately copying repetitive
nucleotide sequences, comprising amplifying said
repetitive nucleotide sequence using said high fidelity
polymerase mutant of claim 22.

39. A method for determining an inherited
mutation, comprising amplifying a gene using a high
fidelity polymerase mutant.

20 40. A method for diagnosing a genetic disease,
comprising correlating the inherited mutation determined
in claim 39 with said genetic disease.

25 41. A method for diagnosing a genetic disease,
comprising amplifying a gene using a high fidelity
polymerase mutant.

42. A method for diagnosing a genetic disease, comprising amplifying a gene using said high fidelity polymerase mutant of claim 22.

43. The method of claim 41, wherein said 5 genetic disease comprises mutations in microsatellite or repetitive DNA.

44. The method of claim 43, wherein said genetic disease is cancer.

45. A method for determining the prognosis of 10 a genetic disease, comprising amplifying said gene in claim 41.

46. The method of claim 41, wherein said polymerase mutant comprises one or more amino acid substitutions selected from the group consisting of 15 Phe667Leu; Asn666Asp; Asn666Ile; Ile665Leu; Leu670Val; Arg660Tyr; Arg660Ser; Gly668Arg; Arg660Lys; Gly668Ser; Gly668Gln; Thr664Ile and Asn666Asp; Ala661Ser and Val669Leu; Ala661Glu, Ile665Thr, and Phe667Leu; and Thr664Pro, Ile665Val and Asn666Tyr.

20 47. A method for randomly mutagenizing a gene, comprising amplifying said gene using a low fidelity polymerase mutant.

48. A method for randomly mutagenizing a gene, comprising amplifying said gene using said low fidelity 25 polymerase mutant of claim 24.

49. The method of claim 48, wherein said polymerase mutant comprises substitution of one or more amino acid residues selected from the group consisting of Ala661, Thr664, Asn666 and Leu670.

5 50. The method of claim 49, wherein said
polymerase mutant comprises one or more amino acid
substitutions selected from the group consisting of
Ala661Glu; Ala661Pro; Thr664Pro; Thr664Asn; Thr664Arg;
Asn666Val; Thr664Pro and Val669Ile; Arg660Pro and
10 Leu670Thr; Arg660Trp and Thr664Lys; Ala662Gly and
Thr664Asn; Ala661Gly and Asn666Ile; Ala661Pro and
Asn666Ile; and Ala661Ser, Ala662Gly, Thr664Ser and
Asn666Ile.

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